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AMINO ACID DISTRIBUTION IN BRAIN AFTER USE OF AMPHETAMINES AND β-PHENYLETHYLAMINE

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SUMMARY: Amino acids in rat brain were assayed after IP injection d-amphetamine or β-phenylethylamine (PEA). Results revealed elevated values when one utilized 2.0-12 mg/kg of d-amphetamine. At 15 mg/kg, however, all amino acids fell into the control range except tryptophan which was elevated nearly threefold, and methionine which showed a tenfold decrease. When utilizing PEA to induce the behavioral changes only methionine is decreased at all concentrations of PEA. Chlorpromazine did not disturb the amino acid distribution induced by amphetamine or PEA. When haloperidol was utilized as the neuroleptic to prevent behavioral change there was a significant increase above control of all the amino acids including homocysteine. The implications of this are discussed in the text.

It has been established that amphetamines or β-phenylethylamine (PEA) can induce a stereotypic behavior pattern in rats, as well as cause disaggregation of polyribosomes and inhibition of protein synthesis (1-3) in the acutely treated rat.

Weiss et al (4) have reported an elevation of tryptophan levels in brain after injection of L-DOPA which has also been shown to disaggregate polyribosomes. Aoki and Siegel (5) exploring causes of brain damage in phenylketonuria found that injection of phenylalanine into young rats induced polyribosomal disaggregation in brain but not in liver, and resulted in significant reduction in brain levels of tryptophan. The polyribosome disaggregation paralleled the depletion of brain tryptophan levels.

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Because of possibly important effects on behavior and protein synthesis in rat brain we have investigated the concentrations of amino acids in whole brain of rats following injection of different doses of d-amphetamine and PEA. The level of amino acids was also determined in the presence of the neuroleptics haloperidol and chlorpromazine which inhibit abnormal behavioral manifestations caused by injecting amphetamine or PEA.

MATERIALS AND METHODS

Animals. CD rats weighing between 300-350 g were divided into 12 groups for treatment purposes. Rats were housed individually at room temperature and maintained on a 12:12 h (7 a.m.-7 p.m.) light/dark schedule with Purina** Laboratory Chow and water continuously available. Four groups (total 25 rats) were injected intraperitoneally with d-amphetamine 2, 3.5, 12, 15 mg/kg, respectively. Nine rats, three per dosage level, were injected (IP) with 25, 50, and 100 mg/kg of PEA, respectively. Two groups of rats received the neuroleptic chlorpromazine 15 mg/kg prior to the injection of either d-amphetamin 15 mg/kg (3 rats) or β-PEA 100 mg/kg (2 rats) while haloperidol 5 mg/kg was given IP to one group before injecting 15 mg/kg of d-amphetamine (2 rats). The neuroleptics were given to protect against the abnormal behavioral manifestations observed with high levels of injected PEA or amphetamine. Two groups (5 rats) were used as controls.

Behavior of the animals was observed for up to 1 h after the injection. Exhibition of stereotypic effects generally occurred within 20 min with amphetamine and 5-10 min with PEA. The animals given the neuroleptics before amphetamine or PEA were observed for 1/2 h. Following this period of observation the animals were decapitated in a cold room (4°C) and the brain rapidly removed and washed with physiological saline until free of blood. The brains were than prepared for amino acid analysis.

Amino acid analysis. The flow chart of the procedure for extraction and concentration of amino acids is shown (Fig. 1). Brains of treated and control animals were pooled for amino acid analysis.

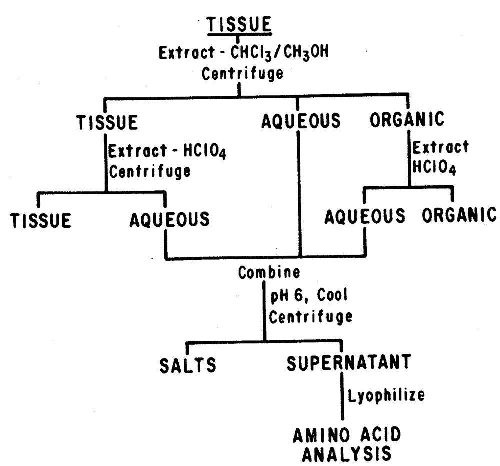

Fig. 1 - Flow Chart of Procedure for Extraction of Amino Acids.

**Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Brains were macerated and extracted in a blender (Waring, Model 91-262) containing 20 ml of Folch's reagent (chloroform/methanol 2:1) at high speed for 1 min. The slurry was centrifuged and the three layers (tissue, chloroform (organic) layer, and upper aqueous phases) were separated. The tissue residue was re-extracted 2 X with 20 ml Folch's reagent and the supernatants combined. The combined chloroform layers were extracted 3 X with 100 ml of 0.6 M perchloric acid and centrifuged. The layers were separated and the tissue re-extracted twice more. Perchloric acid extracts and the initial aqueous phases combined and adjusted to pH 6 with KOH and cooled. The precipitate was washed with ice cold deionized water and the washings were added to the supernatant. The supernatant was concentrated by freeze drying and redissolved in 10 ml of sodium citrate buffer, pH 2.2. Amino acids were separated by using a Beckman Model 119-B and 119-CL A. A. Analyzer pack with resins AA-20 and W-3, respectively. The brains were dissolved in sodium citrate buffer pH 2.2 and separated with the following buffers of pH 3.25, 3.95, and 6.4.

RESULTS

<u>Amphetamines</u>. Nineteen free amino acids were determined in whole rat brain following the injection of varying concentrations of amphetamine. All the amino acids gave evidence of a quadradic ($p < 0.05$) relationship with increased levels of amphetamine except lysine, valine, and proline (Table 1).

Table 1

Effect of IP Administration of d-Amphetamine on Free Amino Acids in Rat Brain

Concentration of Free Amino Acids (nM/g Tissue)

Amino Acids	Control		2 mg		3.5 mg		12 mg		15 mg	
Aspartic acid	1062	927	8671	8489	10,414	10,092	10,398	9375	1976	2004
Tryptophan	9	8	97	67	128	117	108	51	26	--
Threonine	374	349	2329	2204	5830	2754	2629	2386	475	475
Serine	1023	900	7494	7304	7718	7737	8487	7527	1612	1610
Glutamic acid	425	900	4179	4092	4101	4242	5162	4255	1010	908
Proline	765	286	1574	1489	528	1707	1940	--	--	--
Glycine	1009	680	4877	4600	5287	5287	5028	4641	1148	1169
Alanine	1788	--	3468	3413	4503	4235	3439	3572	923	--
Valine	550	950	1874	1650	1902	1879	1549	2066	--	--
Methionine	466	311	396	389	400	938	536	637	45	--
Isoleucine	177	390	808	901	1057	1344	1041	957	196	200
Leucine	426	889	1754	2168	2466	2555	2540	2325	480	487
Tyrosine	1502	4396	6673	6862	7493	6804	9578	9037	3034	3108
Phenylalanine	430	467	1144	1319	1901	1501	1661	1936	500	454
Hydroxylysine	685	855	2204	2142	2413	2024	1072	1097	399	434
Lysine	751	1077	2349	5361	2936	2687	2736	2588	742	781
Histidine	279	401	1057	1074	1257	1128	1188	1079	296	351
Arginine	350	610	1655	1643	1818	1712	1973	1786	300	384
Cysteine-Cystine	955	1258	1004	751	1693	1734	1708	1940	684	680

The administration of 15 mg/kg of amphetamine reversed this trend and depressed the concentration of free amino acids close to or below the level measured in the controls.

At 2 mg/kg all the amino acids were elevated 200%-800% above the control with the exception of methionine where the value remained in the control range as was the case for cysteine-cystine. At 3.5 mg/kg all the amino acids levels increased 150 to 1000% over control except methionine, which was equal to or underwent an increase in one experiment of 100%. The data at the 12 mg/kg level is similar as all amino acids increase 150%-1000% while methionine only increased 50%-100%.

Administration of amphetamine at the 15 mg/kg level caused marked reduction in free amino acids for the most part when compared to administration with lower doses (2-12 mg/kg amphetamine). Aspartic acid, tryptophan, threonine, serine, and glutamic acid are increased only twofold while isoleucine, leucine, tyrosine, alanine, lysine, histamine, and arginine remained at the same level as the control. Three amino acids including alanine, hydroxylysine, and methionine decreased with methionine undergoing a tenfold reduction. At 2-12 mg/kg methionine remains unchanged or undergoes only a modest increase. This has been calculated as 1/4 to 1/20 the increase found in the other amino acids. After injection of 15 mg/kg of amphetamine, however, there is a marked reduction in methionine as seen in Table 1. Whether the amino acids were acid, basic, or neutral did not appear to play a role in the eventual concentration of the amino acids in brain tissue.

PEA. Administration of β-PEA (25, 50, 100 mg/kg) does not significantly effect the free amino acids (Table 2), as did the administration of amphetamine. Only methionine shows evidence of a significant ($p < 0.05$) linear effect due to increased levels of PEA. Methionine is consistently depressed to 40% of the control. Aspartic acid exhibits evidence of similar behavior, but only at a lower significance (<0.10) level. An increase in cysteine-cystine†† occurred after administration of 100 mg/kg of PEA in one experiment.

Table 2

Effect of Administration of β-PEA on Free Amino Acids in Rat Brains

Concentration of Free Amino Acids (nM/g Tissue)

	I				II			
Amino Acids	Control	25 mg/kg	50 mg/kg	100 mg/kg	Control	25 mg/kg	50 mg/kg	100 mg/kg
Lysine	444	197	98	247	186	317	191	482
Histidine	100	53	69	--	--	81	56	--
Arginine	338	249	291	117	441	398	496	452
Aspartic acid	1994	1550	2151	1599	2404	2269	2429	1846
Threonine	3758	2669	2801	3236	4971	5318	5485	4615
Glutamic acid	2876	2330	3069	1895	3934	4907	4347	3761
Glycine	1250	944	1420	1098	1569	1674	1799	1475
Alanine	394	401	779	644	569	757	816	571
Cysteine-cystine	387	124	257	464	399	443	448	1329
Valine	182	155	238	179	218	197	206	169
Methionine	106	60	60	55	117	62	101	71
Isoleucine	115	82	131	108	89	113	139	92
Tyrosine	131	92	130	106	132	120	144	125
Phenylalanine	132	229	132	123	152	678	177	122
Leucine	323	211	328	272	277	278	343	245

Neuroleptics. Injection of neuroleptics to protect the animals against behavioral aberrations usually induced by amphetamine or PEA gave interesting results (Table 3). When chlorpromazine 15 mg/kg was injected prior to amphetamine (15 mg/kg), abnormal behavioral changes were prevented in all cases and the amino acids measured in nm/gm brain tisssue generally were in the range of the controls with the exception of lysine which showed a threefold elevation.

These values were very similar to those noted when chlorpromazine (15 mg/kg) was utilized as the protectant against the manifestations with PEA (100 mg/kg), again with some notable exceptions as shown in Table 3. Conversely when animals were injected with haloperidol (5 mg/kg) doubling of the individual amino acid concentration in nm/gm brain tissue over the controls was noted when compared with the concentration of the amino acids while protecting the animals with chlorpromazine after the injection of either amphetamine or PEA (see Table 3).

Table 3

Amino Acid Distribution After the Use of Neuroleptics to Protect Against Stereotypies

Concentration of Free Amino Acids (nM/g Tissue)

Amino Acid	Amphetamine 15 mg/kg + Control	Amphetamine 15 mg/kg + Chlorpromazine 15 mg/kg	Amphetamine 15 mg/kg + Haloperidol 5 mg/kg	β-Phenylethylamine 100 mg/kg + Chlorpromazine 15 mg/kg
Lysine	186	548 (2.94)	411 (2.21)	181 (0.97)
Histidine	--	--	--	--
Arginine	441	391 (0.89)	873 (1.98)	336 (0.76)
Aspartic acid	2404	2638 (1.10)	4879 (2.03)	2596 (1.08)
Threonine	4971	5496 (1.10)	11,044 (2.22)	5970 (1.20)
Glutamic acid	3934	4724 (1.20)	9347 (2.38)	5104 (1.30)
Glycine	1569	1936 (1.23)	3281 (2.09)	2106 (1.34)
Alanine	569	920 (1.62)	1278 (2.25)	982 (1.73)
Cysteine-cystine	399	482 (1.21)	940 (2.36)	345 (0.86)
Valine	218	178 (0.82)	398 (1.83)	178 (0.82)
Methionine	117	112 (0.96	178 (1.52)	103 (0.88)
Isoleucine	89	119 (1.34)	241 (2.71)	80 (0.90)
Tyrosine	132	138 (1.05)	268 (2.03)	127 (0.96)
Phenylalanine	152	155 (1.02)	291 (1.91)	105 (0.69)
Leucine	277	309 (1.12)	591 (2.13)	570 (2.05)

The number in parenthesis denotes the ratio of the result to controls.

DISCUSSION

We utilized different amounts of amphetamine and PEA as well as protection against the behavioral manifestation of these amines with the neuroleptics chlorpromazine and haloperidol to determine qualitative and quantitative changes of amino acid distribution in whole brain.

In our work we see a threefold increase in tryptophan and a tenfold decrease in methionine when 15 mg/kg of amphetamine is injected IP in a single dose. A significant decrease in methionine and an increase in tryptophan in rat brain following a single IP injection of L-DOPA has also been described by Weiss (4). He suggests that L-DOPA acts on polysomes by limiting the availability of other amino acids such as methionine. This finding is interesting as methionine is essential in the process of protein initiation. The methionine as met-tRNA along with GTP and initiation factor EIf-2 form a ternary complex and promotes the formation of a 40 S complex necessary in the eventual initiation and elongation of the polypeptide chain. It is possible that the decrease in methionine that we see after the use of 15 mg/kg of amphetamine IP is the single most important factor in inhibiting protein synthesis. Baliga and Munro (6) have described a direct inhibitory effect of amphetamine on peptide chain initiation utilizing natural messenger *in vitro* through a step related to formation of the RNA-ribosome complex. They do not elucidate, however, the specific component affected. Nowak and Munro (7) were more specific and thought that amphetamine acts on tRNA acylation in inhibiting protein synthesis. In some of our earlier work utilizing synthetic messenger in the form of Poly U directed incorporation of tritiated phenylalanine, we appeared to be affecting the elongation of polypeptides.

In the present investigation the severe depression of methionine concentration in brain after the use of amphetamine and PEA IP suggests that it is initiation that is affected and may be the major component in the inhibition of protein synthesis when this drug is employed. When utilizing PEA the behavioral manifestations are different than with the use of amphetamine. With PEA we consistently see a Straub tail (the tail is perpendicular to the longitudinal axis of the animal) and abduction of the hind quarters of the animal which is very rarely seen with amphetamine. In previous work we have implicated serotonin as the transmitter probably responsible for these actions (8) while dopamine appears to incite the behavioral event after the use of amphetamines. Another interesting finding was at 100 mg/kg PEA we see nearly a 300% increase in cysteine-cystine (including homocysteine) in one experiment with only a 25% increase in the second experiment. This group of amino acids was not affected by injection of amphetamine.

Sprince in 1969 (9) directed attention to the role of homocysteine, a naturally occurring intermediary metabolite of methionine, as inducing convulsions. While Sprince used methionine to exacerbate schizophreniform behavior in animals, we saw a significant decrease in methionine in animals manifesting stereotypic behavior. One possibility explaining this difference is a rapid change of gradient across the blood brain barrier and cell membrane after a large methionine test load was given, while after the induction of abnormal behavior with amphetamine these changes would be much less rapid allowing more time for adjustment of homostatic mechanisms.

Presently we have no explanation for the very significant elevation of all the amino acids tested when utilizing haloperidol as the neuroleptic protecting against the abnormal behavior after the use of amphetamine. This was not the case when utilizing chlorpromazine as the antipsychotic agent preceding the IP injection of amphetamine. In this case, as shown in the table, all the amino acids tested were comparable to controls except lysine, where we do see a very significant rise of 300%. Utilizing the combination of chlorpromazine and PEA we again see all the amino acids are in the control range. Work is now in progress to elucidate the findings reported in this paper.

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